REMARKS

Status of the Application and Amendments to the Specification and Claims

Applicants thank the Office for entering the Request for Continued Examination under 37 C.F.R. § 1.114 filed March 21, 2003, and the previous claim amendments filed January 22, 2003.

As indicated above, claims 11-13, 16-18, 21-22, 25-26, and 29-40 are currently pending. Of these, claims 11-13, 16-18, and 29-34 are under examination. The Office withdrew claims 21-22, 25-26, and 35-40. The withdrawn "process" claims all depend from "composition" claims 11 or 12, and accordingly, contain all of the elements and limitations of those claims. Therefore, Applicants previously requested rejoinder of the withdrawn claims under the policy of 37 C.F.R. § 1.141 and M.P.E.P. § 821.04 and renew that request here. (See Applicants' remarks filed January 22, 2003, at page 3.)

The Office objects to the specification, contending that it does not provide sufficient antecedent support for claims 11, 16, 29, and 34, that recite the terms "adsorbed" or "adsorption." (Office Action at page 2.) Applicants have amended the specification, according to the Office's request. Applicants have not, however, followed the Office's specific suggestion to replace "absorption" with "adsorption" at every occurrence, as this would require amending the majority of the paragraphs in the specification as well as several of the figures. Instead, Applicants have made amendments at the first occurrence of the terms "absorption ratio" or "absorbed."

In addition, Applicants take this opportunity to correct two inadvertent typographical errors in the paragraph at page 11, line 23, to page 12, line 9. First, the spelling of "immobilization" is corrected. Second, the word "adsorbance" is changed to

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"absorbance of light" in the sentence describing measuring enzyme activity by assaying the decomposition of DL-Mandelonitrile to benzaldehyde using 249.6 nm light.

The amendments to the specification are made solely to speed prosecution, as Applicants note that antecedent support for claims 11, 16, 29, and 34 is already provided in the application, for example at page 2, line 26, to page 3, line 5, and at page 12, lines 10-12, presenting the equation for the "adsorption ratio." (See also Applicants' remarks filed March 21, 2003, describing support for the previous claim amendments.) In addition, these amendments merely re-phrase certain passages in the specification. Hence, these changes do not constitute new matter. (See M.P.E.P. § 2163.07(I) and Applicants' remarks filed March 21, 2003, at pages 2-3.)

Applicants also amend claims 11 and 16 to re-word the phrase "an immobilized enzyme comprising (S)-hydroxynitrile lyase derived from *Euphorbiaceae*, *Poaceae*, or *Olacaceae*" to read "an immobilized *Euphorbiaceae*, *Poaceae*, or *Olacaceae* (S)-hydroxynitrile lyase enzyme." Neither of these changes alters the scope of the pending claims or presents new matter. Indeed, these amendments merely alter the grammatical form of certain phrases. (See M.P.E.P. § 2163.07(I).) Finally, Applicants cancel claims 27 and 28 without prejudice or disclaimer. All of the above amendments are supported by the application as a whole, and Applicants respectfully request their entry.

Rejection under 35 U.S.C. § 112, Second Paragraph

The Office rejects claims 11-13, 16-18, and 29-34, contending that the phrases "derived from" and "an immobilized enzyme comprising (S)-hydroxynitrile lyase," recited in claims 11 and 16, are vague or confusing. (Office Action at page 2.) Solely to speed

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prosecution, Applicants amend claims 11 and 16, changing this phrase to read "an immobilized *Euphorbiaceae*, *Poaceae*, or *Olacaceae* (S)-hydroxynitrile lyase enzyme." Therefore, these rejections are now moot and Applicants respectfully request their withdrawal.

Nevertheless, Applicants reiterate that removing the term "derived from" does not alter the scope or meaning of the pending claims. Moreover, the claimed "*Euphorbiaceae*, *Poaceae*, or *Olacaceae* (S)-hydroxynitrile lyase" enzymes should not be read to exclude enzymes with known modifications, such as those made to enhance expression or activity. For example, proteins are frequently modified during expression, such as by introducing expression tags or fusion peptides. (Incorporating 6-histidine or similar short tags, leader sequences, or fusing the desired protein to an easily expressed peptide or protein, for example, are standard techniques in the art used to improve the expression of a protein. See Exhibit A, providing commercial brochures illustrating several types of protein expression systems involving attachment of peptide tags to the protein of interest.) Proteins are also frequently obtained from non-native sources using genetic engineering technology. In fact, the instant specification describes methods of preparing (S)-hydroxynitrile lyase recombinantly in yeast or *E. coli* cells. (See the specification at pages 3-5.) Accordingly, the claims are *not* limited to enzymes directly isolated from their natural sources, but also encompass enzymes obtained by other methods or expressed in non-native host cells, for example. Further, practitioners seeking to improve enzyme performance or thermal stability may, for example, introduce small modifications into proteins or may eliminate an unnecessary domain. (The Klenow fragment of *E. coli* DNA polymerase I is an example. See Exhibit

B.) Therefore, Applicants' claims should not be read to exclude known or later developed *Euphorbiaceae*, *Poaceae*, or *Olacaceae* (S)-hydroxynitrile lyase enzymes that incorporate such developments and that are available to those of ordinary skill in the art.

Rejection under 35 U.S.C. § 102(b)

The Office also rejected claims 11-13, 16-18, and 29-34 as allegedly anticipated by Andruski et al. (U.S. Patent No. 5,177,242; "Andruski"). (Office Action at page 3.) The Office contends that Andruski teaches "an immobilized enzyme comprising (S)-hydroxynitrile lyase derived from plants such as *Poaceae*, wherein the enzyme is immobilized on a carrier comprising a porous inorganic material." (*Id.*, referring to col. 4, lines 7-16, and Example 1 of Andruski) Further, the Office contends that Andruski teaches an immobilized sorghum bicolor (S)-hydroxynitrile lyase enzyme. (*Id.*) Applicants respectfully traverse this rejection.

In order for a single reference to anticipate a claim, that reference alone must contain, either expressly or inherently, each and every element of the claim. M.P.E.P. § 2131; *Verdegaal Bros. v. Union Oil Co. of California*, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987). Applicants respectfully submit that Andruski does not recite each element of Applicants' claims either expressly or inherently, and hence, cannot anticipate any of the pending claims. Thus, as demonstrated below, this rejection is not based on a *prima facie* case of anticipation.

Applicants respectfully submit that the Office has not considered that claims 11 and 16 recite that the enzyme is "***adsorbed on***" the carrier, not just "immobilized on" it. In contrast, Andruski teaches a distinct method that employs a covalent cross-linker. As

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set forth below, "adsorbed" enzymes interact with carriers differently from covalently cross-linked enzymes.

In support of the distinction between "adsorption" and cross-linking, Applicants direct the Office's attention to the article by R.A. Messing, submitted to the Office in January, 2003. ("Adsorption and Inorganic Bridge Formations," *Meth. Enz.*, Vol. XLIV, pp. 148-161 (1976).) Messing describes "adsorption," in the context of protein-carrier attachment, as "the adhesion of an enzyme to the surface of a carrier that has ***not been specifically functionalized for covalent attachment***." (Messing at p. 149, emphasis added.) Thus, Messing specifically distinguishes the "adsorption" method that Applicants claim from covalent cross-linking methods such as that of Andruski. Further, the article points out that interactions between carriers and "adsorbed" enzymes are often entirely non-covalent, involving ionic (charge-charge or salt-bridge) and hydrogen bonding interactions. (*Id.* at pp. 149-153.) In addition, while covalent bonds can form under some circumstances of adsorption, they form directly between the carrier material and the protein. (*Id.* at pp. 152-3) In contrast, cross-linking methods like that of Andruski necessarily yield not only covalent attachments, but also require a third molecule (the cross-linker) to act as a bridge between the carrier and the protein.

In Andruski's method, membranes to which enzymes will be attached are first incubated with a cross-linker such as polyethyleneimine (PEI), and then with glutaraldehyde.[1] (*See, e.g.*, Andruski at col. 3, lines 55-61; col. 4, lines 5-16; col. 4, line 56, to col. 5, line 8; col. 5, line 58, to col. 6, line 25; and Example 1.) PEI and glutaraldehyde serve as a bridge to covalently link the protein and carrier. (*Id.* at col. 5,

[1] The PEI cross-linking method is the only method that is exemplified in Andruski.

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line 58, to col. 6, line 25, and Example 1.) Thus, Andruski specifically functionalizes its membranes for covalent attachment of the enzyme, a method that Messing points out is ***not*** "adsorption." (See previous paragraph.) Further, Andruski's enzyme is necessarily covalently bound to the membrane through the PEI-glutaraldehyde bridge. In contrast, Applicants' "adsorption" method may result in entirely non-covalent interactions between the carrier and enzyme and does not require any bridging molecule. Thus, Applicants' claimed immobilized enzyme compositions are structurally different from those of Andruski.

Because Andruski does not teach, either explicitly or inherently, an enzyme that is "adsorbed on" a carrier, Andruski cannot anticipate any of Applicants' claims. Hence, Applicants respectfully request the withdrawal of this rejection.

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CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application, and the timely allowance of claims 11-13, 16-18, and 29-34. Applicants also request the rejoinder of withdrawn claims 21-22, 25-28, and 35-40 to this application upon allowance of claims 11-13, 16-18, and 29-34.

Please grant any extensions of time required to enter this response and charge any required fees not submitted herewith to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: August 18, 2003

By: _____
Elizabeth A. Doherty
Reg. No. 50,894

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Protein Expression Systems

Expression of Recombinant Proteins

Your choice of expression system depends on the downstream application of the purified protein.

Bacteria

Bacterial expression systems are the most popular means of expressing recombinant proteins. Bacteria can be easily transformed with versatile expression constructs and can be easily selected for positive colonies. Bacteria grow rapidly and express high levels of recombinant proteins. In addition, the bacterial genome is relatively well-characterized. BD Biosciences Clontech offers several bacterial expression systems: HAT™, PROTet™, and Creator™-compatible 6xHN-Vectors. However, when expressing in bacterial systems many recombinant proteins become insoluble and are trapped in inclusion bodies. Another drawback is that proteins expressed in bacteria will not have any eukaryotic post-translational modifications.

Yeast

Yeast expression systems are a good alternative when a bacterial expression system will not be adequate because you get expression levels ranging up to several milligrams per liter of culture with most of the eukaryotic post-translational modifications. Some recombinant proteins that are insoluble when expressed in bacteria will be soluble when expressed in yeast because the protein processing is more complex in yeast. BD Biosciences Clontech offers the YEASTMAKER™ Yeast Transformation System and the YEASTMAKER™ Yeast Plasmid Isolation Kit, as well as many types of yeast media and MATCHMAKER Yeast Two-Hybrid System 3. One drawback to using yeast expression; however, is that yeast cells may acidify the culture medium and both the cells and the medium may contain compounds that affect binding of His-tags to the resin. Also, transfecting and lysing yeast cells can be challenging processes.

Baculovirus

Baculovirus vectors are a viral system for expressing proteins in insect cells. Baculovirus systems rely on the principle that baculovirus infects and multiplies in cultured insect cells. This is advantageous because insect cells also recognize most mammalian protein-targeting sequences. Thus, they can express a variety of proteins. Insect cells can also perform many of the post-translational modifications performed in mammalian cells. Recombinant proteins can be produced either within the cells or secreted into the culture medium. We offer the BacPak™ Baculovirus Expression System, for efficient production of high yields of recombinant protein. Despite its many advantages, baculovirus expression can be challenging because baculovirus vectors are sometimes difficult to generate and use in infecting cells. Also, insect cells grow more slowly than bacterial and yeast cells.

Protein Expression Systems...*cont.*

Mammalian cells

Mammalian cells usually provide the best system for generating recombinant eukaryotic proteins because they produce necessary post-translational modifications and recognize the same synthesis and processing signals found in the original organism. We offer several mammalian expression systems, including multiple plasmid-based systems, inducible Tet-On™ and Tet-Off™ Expression Systems, the adenoviral Adeno-X™ System, the inducible adenoviral Adeno-X™ Tet-On™ and Tet-Off™ Systems, and multiple Retroviral Expression Systems.

Mammalian systems can have several drawbacks. The expression levels are sometimes low and mammalian cells grow much more slowly than bacteria or yeast. In addition, it is expensive to grow mammalian cells in large quantities. Mammalian cell transfections are generally less efficient, which contributes to lower overall expression levels in those systems.

Table IV: Comparison of Expression Systems

Expression System	Advantages	Disadvantages	BD Biosciences Clontech Vectors
Bacteria	Easy, fast, can produce a large quantity of protein rapidly	No post-translational modifications, no phosphorylation or glycosylation	PROTet, HAT, 6xHN Vectors
Yeast	Some post-translation modifications, faster than mammalian	Handling may be difficult	MATCHMAKER Vectors
Baculovirus	Large quantities of proteins, some post-translational modifications, secreted and tagged forms	Difficult to handle large vectors	BacPak Vectors
Mammalian	Post-translational modifications	Slow, more complex, expensive	Tet, Retroviral, Adenoviral, CMV Vectors

Protein Expression Systems...*cont.*

Histidine-tag expression systems

Widespread application of recombinant genetic technologies has fostered the production of recombinant proteins containing polyhistidine tags on either the N- or C-terminus (Hochuli *et al.*, 1987; Hochuli *et al.*, 1988). Histidines exhibit highly selective coordination with certain transition metals and have great utility in IMAC. Under conditions of physiological pH, histidine binds by sharing electron density of the imidazole nitrogen with the electron-deficient orbitals of transition metals (Figure 2). Although three histidines may bind transition metals under certain conditions, six histidines reliably bind transition metals in the presence of strong denaturants such as guanidinium (Hochuli *et al.*, 1987). Such protein tags are commonly referred to as "6xHis," "hexaHis," or "(His)6." However, since development and widespread use of this tag, sometimes it was found that the 6xHis tag affects the solubility of expressed proteins. Other tags are now available that exploit the histidine binding, yet have better solubility characteristics.

Histidine tags

The HAT™ sequence (patent pending) is a novel IMAC affinity tag derived from a unique natural protein sequence in chicken lactate dehydrogenase. This tag contains six histidines unevenly interleaved by other amino acid residues (Table V) and does not have the excessive positive charge characteristic of the commonly used 6xHis tag. Thus, HAT-fusion proteins have better solubility and similar affinity towards immobilized transition metal ions and zinc. In addition, HAT-fusion proteins can be adsorbed in the absence of imidazole at neutral pH. As a result, the alkaline proteases present in cell lysates are less active, and therefore most HAT-tagged proteins are more stable.

The 6xHN tag is a histidine-rich peptide that has similar solubility and binding characteristics to 6xHis (see page 5), but is more useful than 6xHis when purifying high molecular weight proteins. Generally, it is more difficult for a resin to bind a high molecular weight protein than a low molecular weight protein because the bulk of the larger proteins can interfere with the resin's ability to bind to the histidine tag. The 6xHN tag tends to be more exposed on the surface of the protein than 6xHis, so it is easier for TALON resin to bind the 6xHN tag.

Table V: Histidine Tags

Tag	Amino acids
6xHis	His –His –His –His –His –His
6xHN	His –Asn –His –Asn –His –Asn –His –Asn –His –Asn–His –Asn
HAT	Lys –Asp –His –Leu –Ile –His –Asn –Val –His –Lys –Glu –His –Ala –His –Ala –His –Asn–Lys

Protein Expression Systems...*cont.*

Histidine-tag Expression Systems

The PRO™ Bacterial Expression System is based on a set of tetracycline-inducible prokaryotic expression vectors that allow tightly controlled gene expression over a broad range of induction levels. These vectors combine features that let you:

- Precisely control expression. The PRO Vectors produce extremely low background, high expression levels, and precise expression in response to the level of tetracycline inducers.
- Reduce expression problems. Controlled expression of a given protein greatly reduces the formation of insoluble inclusion bodies. It also allows the expression of toxic or growth-inhibitive proteins.
- Purify expressed proteins quickly and easily. The PROTet 6xHN Vectors encode an N-terminal polyhistidine affinity tag. The 6xHN tag allows proteins to be easily and efficiently purified. All PROTet Vectors contain an enterokinase (EK) site so that the tag can be removed from the protein of interest by proteolytic cleavage.
- Modify the vectors to suit your expression needs. The design of PRO Vectors allows them to be easily customized. Each vector consists of three main functional units separated by three unique restriction sites, allowing you to construct new vectors by substituting for any of the three modules independently.

The PROTet Vectors are available in a Creator™-compatible format. The Creator System is our comprehensive, integrated platform enabling gene transfer from a donor vector into multiple expression acceptor vectors without cloning. With Creator-compatible PROTet Vectors, you can easily express your target gene in a highly inducible bacterial expression system. For more information on the Creator System, visit www.clontech.com.

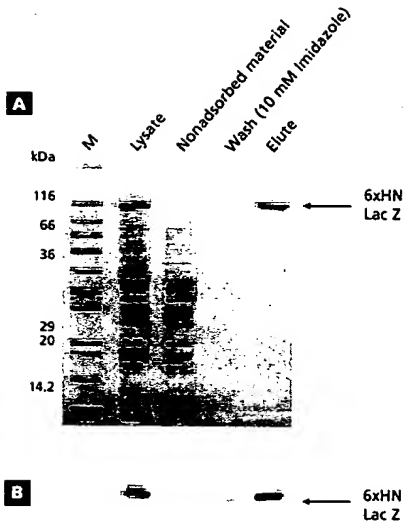

Figure 16. Bacterially expressed 6xHN-tagged protein purified with TALON™ Superflow. Panel A. Coomassie stain of 6xHN-LacZ purification fractions. **Panel B.** Western blot of fractions detects fusion with the 6xHN Polyclonal Antibody. M= molecular weight marker.

Protein Expression Systems...*cont.*

The HAT™ System

The HAT™ Protein Expression & Purification System (patent pending) provides a more convenient and efficient way to express and purify proteins. The HAT Vectors encode a novel polyhistidine epitope tag discovered in avian species that enables purification of protein expressed in bacteria under the mild conditions of neutral or physiological pH. The tag is based on a natural poly-histidine peptide, so it is less likely to result in inclusion body formation. The tag is also longer than 6xHis, which may be beneficial for expressing and purifying high molecular weight proteins because the HAT tag tends to be located on the outside of high molecular weight proteins. Therefore, resin can bind the histidine residues more easily than when they are buried within the structure of the protein. In concert with TALON Resin, the HAT Vectors facilitate simplified protein purification under either native or denaturing conditions.

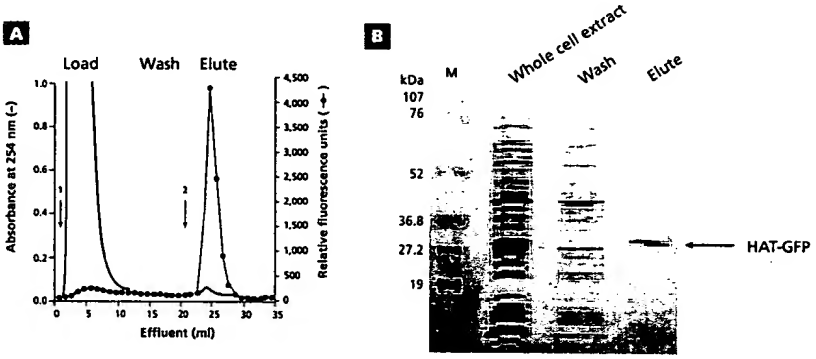

Figure 17. FPLC purification of HAT-tagged protein with TALON™ Superflow. *E. coli* cells were extracted in 50 mM sodium phosphate, 0.3 M NaCl, 5 mM imidazole, pH 7.0 and eluted in 150 mM imidazole. **Panel A.** Cell lysate was purified with a TALON Superflow column. **Panel B.** SDS-PAGE analysis of the procedure. M=molecular weight markers.

HAT Protein Purification

The HAT System offers advantages for native protein purification compared to 6xHis-tag purification protocols that require the use of alkaline buffers (pH 8). Purification at neutral pH is more efficient due to the reduction in binding and elution of impurities, such as non-His-tagged proteins. In addition, purification at neutral pH decreases the activity of basic proteases and generally results in higher protein stability. For proteins that exhibit lower solubility, the HAT System is also suitable for purification under denaturing conditions.

Proteins can be expressed with the HAT tag from pHAT, which contains an enterokinase cleavage site to obtain the native protein. For protein purification, the HAT tag is ideal for use with TALON resin, which selectively binds His-tagged (or HAT-tagged) proteins. TALON requires only low stringency washes to remove nonspecific proteins. We do not recommend using the HAT tag with nickel-based resins because high stringency conditions are required to remove nonspecifically-bound proteins from Ni-NTA columns. Additionally, the HAT tag can easily be incorporated into any other expression vector using PCR cloning.

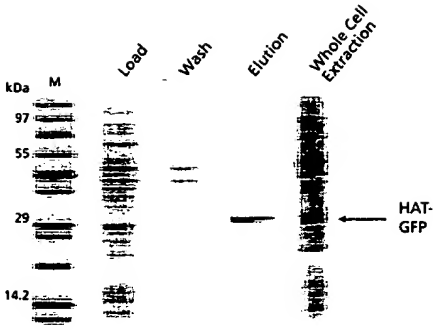

Figure 18. Batch purification of HAT-GFP by pH gradient. SDS-PAGE analysis of protein purification using pH 6.0 elution buffer. Sample was loaded in loading/washing buffer (50 mM sodium phosphate, 0.3 M NaCl, pH 7.0). After washing, the protein was eluted with the same buffer at pH 6.0. M=size markers.

Table VI: The HAT™ System

Features of the HAT™ System	Benefits
Longer tag	Best for high molecular weight proteins
Evenly distributed charge throughout the tag	Higher solubility
Based on unique natural sequence	Lower probability of toxicity to the host cell
Purification at physiological pH	No damage to the target protein

Affinity® Protein Expression and Purification System

- pCAL vectors derived from T7 RNA polymerase-based pET vectors‡‡
- Novel purification tag combines small size, gentle elution and high-affinity binding
- Easy tag removal resulting in native protein
- System consists of vectors, competent cells and resin

Applications

- High-level protein expression
- One-column purification

Best System for High-level Protein Expression

The Affinity® protein expression and purification system provides a simple, gentle and effective method for high-level protein expression and purification. The system includes pCAL expression vectors containing a calmodulin-binding peptide tag and calmodulin affinity resin (available separately). For rapid, sensitive detection of tagged fusion proteins, Stratagene offers the Affinity® CBP protein detection kit.

Easy Purification

The T7 RNA polymerase-based pCAL vectors express cloned proteins as fusions with the calmodulin-binding peptide (CBP) tag. The CBP-tagged proteins can be purified to near homogeneity after only one pass through calmodulin resin. The CBP tag binds to calmodulin resin in the presence of low concentrations of calcium and is eluted in the presence of 2 mM EGTA at neutral pH. It is an excellent alternative to the 6xHis affinity tag that requires harsh elution conditions. The small 4-kDa CBP tag may have less effect on the protein of interest than larger tags such as the 26-kDa GST affinity tag. Expressed proteins also include a thrombin or enterokinase‡‡‡ protease cleavage site, depending on the vector, for optional CBP tag removal.

pCAL Vectors

- High levels of expression
- Tight regulation
- Proteins can be expressed as C- or N-terminal fusions
- In vitro ^{32}P-labeling of expressed proteins
- pCAL-n-FLAG vector for LIC cloning

Selection

Ampicillin resistance in *E. coli*

CLONING SITES

pCAL-n vector: *BamH* I, *Sma* I, *EcoR* I, *Xba* I, *Nco* I, *Sal* I, *Xho* I, *Sac* I, *Hind* III
pCAL-kc vector: *Nco* I, *Nhe* I, *BamH* I
pCAL-c vector: *Nco* I, *Nhe* I, *BamH* I

PROMOTER:

T7/lacO promoter

Highest Levels of Pr tein Expression

Stratagene's pCAL vectors are derived from the T7 RNA polymerase-based pET-11 series vectors to achieve exceptionally high levels of protein expression. All five of the pCAL vectors contain the *T7/LacO* promoter and a plasmid-borne copy of the *lacI*^{q} gene to allow tight repression in *E. coli*. In the presence of IPTG, T7 RNA polymerase is expressed in the *E. coli* BL21(DE3) or BL21(DE3) pLysS host cells. The T7 RNA polymerase binds to, and transcribes from, the T7 promoter on the pCAL vector. High-level translation utilizes the strong T7 gene 10 ribosome-binding site. Transcription and translation are highly effective and after only a few hours, the target protein may constitute the most abundant cellular protein. Using the pCAL vectors, recombinant proteins can be expressed as C- or N-terminal fusion proteins to the CBP affinity tag. The CBP portion of the expressed proteins may be labeled in vitro with cyclic AMP-dependent protein kinase and γ-^{32}P. The presence of the kemptide sequence expressed from the pCAL-kc vector allows in vitro protein labeling in experiments where the CBP tag will be removed.

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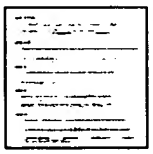

Multiple Cloning Site Sequences

Vector Maps

Affinity Purification
The JNK gene was cloned into the pCAL-n vector and transformed into BL21(DE3) pLysS cells.
Cultures were induced with IPTG and lysed.
Lane 1: Input lysate, Lane 2: Flowthrough lysate, Lane 3: Eluate

Vector Details

pCAL-c

Sequence	Restriction Sites	Vector Map

pCAL-kc

Sequence	Restriction Sites	Vector Map

pCAL-n

Sequence	Restriction Sites	Vector Map

pCAL-n-EK

Sequence	Restriction Sites	Vector Map

pCAL-n-FLAG

Sequence	Restriction Sites	Vector Map

Found 11 Catalog Items | Displaying 1 - 5

Amount	Catal g#	Stock	Price in US DOLLARS

pCAL-c Affinity® Pr tein Expression System
20 µg pCAL-c vector (1.0 µg/µl), 5 x 0.2-mL BL21 (DE3) cells, thrombin, Calmodulin Affinity Resin, EGTA (100 mM)

1 kit	204301		510.00	ADD TO

pCAL-kc Affinity® Protein Expression System

20 µg pCAL-kc vector (1.0 µg/µl), 5 x 0.2-mL BL21 (DE3) cells, thrombin, Calmodulin Affinity Resin, EGTA (100 mM)

1 kit	204300		510.00	ADD TO

pCAL-n Affinity® Protein Expression System

20 µg pCAL-n vector (1.0 µg/µl), 5 x 0.2-mL BL21 (DE3) cells, thrombin, Calmodulin Affinity Resin, EGTA (100 mM)

1 kit	204302		510.00	ADD TO

pCAL-c Vector

Derived from pET-11d, Rrcombinant proteins expressed as C-terminal fusion to the CBP affinity tag, Thrombin-cleavage site is immediately downstream of *BamH* I cloning site

20 µg pCAL-c vector, *E.coli* XL1-Blue strain	214301		270.00	ADD TO

pCAL-kc Vector

Derived from pET-11d, 9 amino acid kemptide sequence is between *BamH* I and thrombin-cleavage site, Kemptide sequence allows proteins to be labeled with PKA and $\gamma^{32}P$ where the CBP tag has been removed

20 µg pCAL-kc vector, *E.coli* XL1-Blue strain	214300		270.00	ADD TO

next 5 items ▷ shopping cart

Manuals

204300 Affinity® Protein Expression System (with pCAL-kc)
204301 Affinity® Protein Expression System (with pCAL-c)
204302 Affinity® Protein Expression System (with pCAL-n)
214300 Affinity® Protein Expression Vector pCAL-kc
214301 Affinity® Protein Expression Vector pCAL-c

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ESP® Yeast Protein Expression and Purification System

- Eukaryotic protein expression in yeast *Schizosaccaromyces pombe*
- Capable of many posttranslational modifications
- Single-column purification using GST tag§§
- Tight regulation of protein expression
- Provides most reagents necessary for high-level protein expression and purification of your protein of interest

Cloning Sites

pESP-1: *BamH* I, *Xma* I and *Sma* I sites
pESP-2: *BamH* I, *Bgl* II, *Nhe* I, Sph I and *Srf* I sites
pESP-3: *Asc* I, *BamH* I, *Nde* I and *Srf* I sites

Selection

Ampicillin resistance in *E. coli*, LEU selection in yeast

Promoter

nmt1 promoter of *S. pombe*

Origin

ars fragment in yeast, ColE1 in bacteria

Advanced Host with Unique Capabilities

The ESP® yeast protein expression and purification system uses the yeast *Schizosaccharomyces pombe* as the expression host and the glutathione S-transferase (GST) peptide as the protein purification tag. This system provides an easy alternative to protein production in *E. coli*. Proteins expressed in *E. coli* may lack proper biological function and antigenicity because of the absence of eukaryotic posttranslational modifications. *S. pombe* is a single-cell eukaryotic organism with properties similar to higher eukaryotic organisms. These properties, such as chromosome structure and function, cell-cycle control, RNA splicing and codon usage, make *S. pombe* ideal for the production of eukaryotic proteins. Also, eukaryotic proteins expressed in *S. pombe* are more likely to be folded properly, which improves the specific activity and can eliminate protein insolubility problems found in *E. coli*.

Rapid One-Column Purification

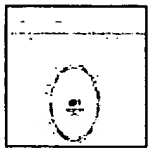
The GST affinity tag allows for rapid, near homogeneous, single-column purification of proteins expressed from the pESP vectors. N- or C-terminal tags are available, depending on the pESP vector. After expression in *S. pombe*, the GST fusion protein can be purified from crude cell extracts using a single-column purification procedure based on the selective affinity of the GST peptide for glutathione immobilized on the GST resin. Purification of the GST fusion proteins to 90% homogeneity is easily achieved using this procedure. After purification, the GST affinity tag may be removed by proteolytic cleavage with enterokinase‡‡‡ or thrombin. Thrombin cleavage leaves the FLAG® epitope tag‖ ‖ in the recombinant protein, allowing further analysis when using the pESP-1 or pESP-2 vectors.

Tightly Controlled Expressi n

Protein expression from the pESP vectors is controlled by the *nmt1* (no message

in thiamine) promoter. The p_{nmt1} is strictly regulated by the concentration of thiamine in the media. This promoter is active in the absence of thiamine, allowing downstream transcription of genes under its control. In the presence of greater than 0.5-µM thiamine, the promoter is turned off. The induction ratio of the promoter is approximately 300-fold. The *nmt1* promoter is more than 80% stronger than the commonly used yeast *adh* promoter. Media for induced and non-induced growth of the SP-Q01 *S. pombe* host is included with the ESP systems.

Click For Full Size



Vector Details

pESP-1

Sequence | Restriction Sites | Vector Map

pESP-2

Sequence | Restriction Sites | Vector Map

pESP-3

Sequence | Restriction Sites | Vector Map

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Amount	Catalog#	Stock	Price in US DOLLARS	
ESP® Yeast Protein Expression and Purification System pESP-1 vector, pESP-1 control vector, forward and reverse PCR/sequencing primers, XL1-Blue supercompetent cells, *S. pombe* strain, YES and EMM Medium, Acid Washed Glass Beads, GST Affinity Resin				
1 kit	251600		575.00	ADD TO
pESP-1 Vector Vector, Forward and reverse PCR/sequencing primers, *S. pombe* strain				
vector	217420		270.00	ADD TO
pESP-2 Plasmid				

Vector, Forward and reverse PCR/sequencing primers, *S. pombe* strain

217444 270.00 ADD TO

pESP-3 plasmid

Vector, Forward and reverse PCR/sequencing primers, *S. pombe* strain

217445 270.00 ADD TO

SP-Q01 Yeast Strain

S. pombe glycerol stock

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Manuals

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217444	pESP-2 Supercoiled Plasmid
217445	pESP-3 Supercoiled Plasmid
251600	ESP Yeast Protein Expression

References

1. Maundrell, K. (1990) *J. Biol. Chem.* 165: 10857-10864.
2. Forsburg, S. (1993) *Nucleic Acids Res.* 21: 2955-2956.
3. Smith, D.B., and Johnson, K.S. (1988) *Gene.* 67: 31-40

Licensing Information

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DNA Polymerase I, Large Fragment (Klenow)

#M0210S 200 units **$50 (USA)**
#M0210L 1,000 units **$200 (USA)**
for high (10X) concentration, order #M0210M (1,000 units)

- Isolated from a recombinant source
- Specific Activity: 20,000 units/mg
- Dideoxy sequencing
- Generates probes using random primers
- Creates blunt ends
- Supplied with 10X Reaction Buffer

Description: DNA Polymerase I, Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3′-->5′ exonuclease activity, but has lost 5′-->3′ exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5′ termini.

Source: A genetic fusion of the *E. coli pol* A gene, that has its 5′ -->3′ exonuclease domain genetically replaced by maltose binding protein (MBP). Klenow Fragment is cleaved from the fusion and purified away from MBP. The resulting Klenow fragment has the identical amino and carboxy termini as the conventionally prepared Klenow fragment.

Applications

- DNA sequencing by the Sanger dideoxy method (2)
- Fill-in of 5′ overhangs to form blunt ends (3)
- Removal of 3′ overhangs to form blunt ends (3)
- Second strand cDNA synthesis
- Second strand synthesis in mutagenesis protocols (4).

Reaction Buffer: 1X EcoPol Buffer
[10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 7.5 mM dithiothreitol].
Supplement with dNTPs (not included).

Klenow Fragment is also active in any restriction enzyme reaction buffer and T4 DNA Ligase reaction buffer when supplemented with dNTPs.

Note on Use: Protocol for blunting ends by 3′ overhang removal and 3′ recessed end fill-in: DNA should be dissolved in any 1X restriction enzyme NEBuffer or 1X EcoPol Reaction Buffer supplemented with 33 µM each dNTP. Add 1 unit Klenow per microgram DNA and incubate 15 minutes at 25°C. Stop reaction by adding EDTA to a final concentration of 10 mM and heating at 75°C for 20 minutes. CAUTION: Elevated temperatures, excessive amounts of enzyme,

failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3′-->5′ exonuclease activity of the enzyme.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases. Each lot is functionally tested in "fill-in" reactions. Additionally, each lot is analyzed by SDS polyacrylamide gel electrophoresis for the presence of detectable (less than 1%) uncleaved DNA Polymerase I.

Unit Definition: One unit is defined as the amount of enzyme required to convert 10 nmols of dNTPs to an acid-insoluble form in 30 minutes at 37°C.

Unit Assay Conditions: 1X EcoPol Reaction Buffer, 33 µM dNTPs including [^{3}H]-dTTP and 70 µg/ml denatured herring sperm DNA.

Concentration: 5,000 and 50,000 units/ml.

Storage Conditions: 100 mM KPO_4 (pH 6.5), 1 mM dithiothreitol and 50% glycerol. Store at -20°C.

Heat Inactivation: 75°C for 20 minutes.

References:

1. Jacobsen, H. et al. (1974) *Eur. J. Biochem.* 45, 623-627.
2. Sanger, F. et al. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* , second edition, pp. 5.40-5.43, Cold Spring Harbor Laboratory, Cold Spring Harbor.
4. Gubler, U. (1987) *Methods Enzymol.* 152, 330-335.